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THE APPEARANCE OF PHOSPHOLIPASE ACTIVITY IN THE HUMAN MACROPHAGE-LIKE CELL LINE U937 DURING DIMETHYL SULFOXIDE INDUCED DIFFERENTIATION

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Summary: The human histiocyte cell line, U937, with monocyte characteristics, can be induced to differentiate into macrophage-like cells when exposed to growth medium containing 1.5% DMSO. Following three days of exposure, DMSO-treated but not control U937 cells can be stimulated to release endogenous arachidonic acid from their phospholipids. Maximum release of the unsaturated fatty acid occurs with 10 uM calcium ionophore in the presence but not in the absence of exogenously added calcium ion. In addition, DMSO-treated but not control U937 cells exhibit phospholipase activity when exposed to human IgG and then anti-human immunoglobulin. These data suggest that with respect to arachidonic acid metabolism U937 cells differentiate into functional macrophage-like cells when exposed to DMSO.

A human hematopoietic cell line (U937) was established from a patient with generalized histiocytic lymphoma by Sundstrom and Nilsson in 1976 (1) and has been successfully maintained in suspension culture. These cells appear to represent immature monocytic cells (1) and, when induced to differentiate, undergo a stepwise morphological and functional maturation similar to that described for differentiating normal monocytic cells (2,3). Further research has indicated that the U937 cell line can be induced to differentiate into chemotactic peptide sensitive macrophage-like cells by exposure to medium conditioned by lectin-stimulated lymphocytes, to 1.3% dimethyl sulfoxide or to 1 mM dibutyryl cAMP (2,4-6). In addition to chemotaxis, the

The abbreviations used are: HHT, 12-hydroxy-5,8,10-heptadecatrienoic acid; 5-HETE, 5-hydroxy-6,8,11,14-iocasatetraenoic acid; LTB_4, 5S,12R-dihydroxy-6,8,10,14-icosatetraenoic acid, leukotriene B_4.

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abilities to produce superoxide anion, to release lysozomal enzymes and to perform antibody dependent cellular cytolysis have been induced by these agents (4,7). Therefore, the U937 cell line, although neoplastic, appears to be a useful model of various biological and biochemical parameters of normal human monocyte-macrophage function.

Since normal macrophages metabolize endogenous arachidonic acid when stimulated with the appropriate agonists (8-15), it was of interest to investigate the ability of U937 cells to release the unsaturated fatty acid from their endogenous phospholipids. Moreover, the expression of this phospholipase activity could serve as a marker of differentiation as has been previously observed with the promyelocytic leukemia cell line HL60 following DMSO-induced differentiation to a granulocyte-like cell (16). This report describes the appearance of calcium ionophore and antibody complex stimulatable release of arachidonic acid from endogenous phospholipids in U937 cells during DMSO-induced differentiation. The induction of such an activity is consistent with the previously reported increase in functional responses of these cells following growth in DMSO containing medium (4).

MATERIALS AND METHODS: Cells. The U937 cells were a generous gift from Dr. H. S. Koren, Duke University. The U937 cell line was cultured in RPMI 1640 with 10% heat inactivated fetal bovine serum (lot 100389, Sterile Systems, Logan UT), penicillin (50 units/ml), streptomycin (50 ug/ml), 1% MEM non-essential amino-acids, 1% sodium pyruvate and 1% L-glutamine (all from Gibco, Grand Island, NY). Cell cultures were split so that the cell density was maintained between 2×10^5 and 2×10^6 cells/ml. Cells were induced to differentiate by the addition of DMSO to a final concentration of 1.5% to the above culture medium.

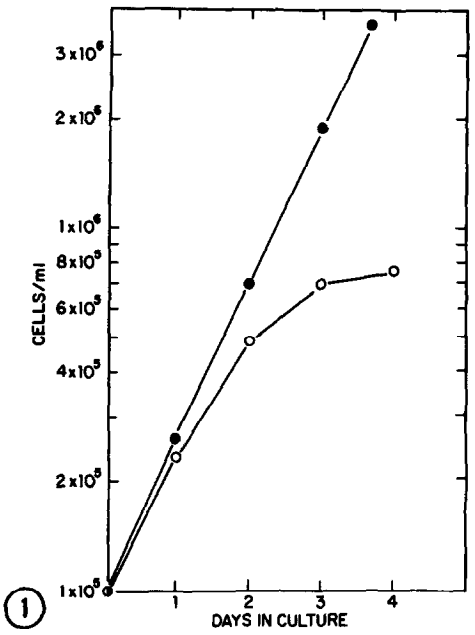
Phospholipase Assay. Cell cultures were incubated overnight with [1-^{14}C]arachidonic acid (Amersham, 55 uCi/umole) at a concentration of 2 uM (0.1 uCi/ml). Labelled cells were harvested by centrifugation and washed twice with 50 mM Tris-HCl buffer, pH 7.4, containing 100 mM NaCl. Washed cells were resuspended in 50 mM Tris-HCl buffer, pH 7.4, containing 100 mM NaCl, 1 mM $CaCl_2$, and 1 mM glucose. The cell suspensions (1 ml) were incubated at 37° for various times as indicated in the figure legends. Reactions were terminated by the addition of 2.4 ml chloroform:methanol (1:1 v/v) and 10 ul of 10% formic acid. The samples were cooled in ice and centrifuged. The organic

layer was withdrawn and evaporated to dryness under a steady stream of N_2. The dry extract was then redissolved in a small volume of chloroform:methanol (1:2 v/v) and spotted on silica thin layer plates (sil G25, without gypsum, Brinkmann). Chromatograms were developed with an ascending solvent system composed of the upper layer of ethylacetate:iso-octane:glacial acetic acid:water (90:50:20:100 v/v/v/v). Labelled products were located by autoradiography and the appropriate regions of the chromatograms were scraped and counted in a liquid scintillation counter. Products were identified by co-chromatography with authentic standards. All assays were in duplicate and have been repeated at least twice. Values are plus or minus 10% of the mean. Since negligible metabolism of released arachidonic acid occurred, phospholipase activity was determined by measuring the free arachidonic acid produced upon stimulation.

RESULTS: U937 cells grown in medium containing 1.5% DMSO cease to divide after approximately three days. As illustrated in Figure 1, while control cultures of U937 cells double in density approximately every seventeen hours, DMSO-treated cells slow their rate of division by the second day of exposure and cease to divide by the third. Viability, as measured by trypan blue exclusion, however, is not significantly affected (data not shown).

This halt in cell growth and division is reflected by an increased ability of DMSO-treated cells to release arachidonic acid from endogenous phospholipids. As illustrated in Figure 2, while both control and DMSO-treated cells readily incorporate arachidonic acid from medium into their phospholipids, control U937 cells do not release the unsaturated fatty acid when exposed to the calcium ionophore A_{23187}. However, cells grown in the presence of 1.5% DMSO for at least three days express this phospholipase activity (Fig. 2). The induction of this enzymatic activity corresponds to the cecession of growth observed in DMSO-treated cultures (Fig. 1).

The calcium ionophore stimulated release of arachidonic acid from DMSO treated but not control U937 cells is concentration dependent. As shown in Figure 3, DMSO-treated cells respond to A_{23187} with maximal arachidonic acid release occurring at a



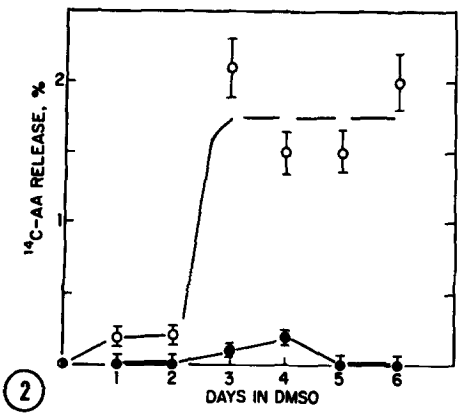


Fig. 1. Growth of U937 cells in DMSO containing medium. U937 cells were grown in suspension culture in the absence (●) or presence (o) of 1.5% DMSO as described in Materials and Methods. The number of cells in the culture was measured daily. As determined by trypan blue exclusion, viability was greater than 95% in control and DMSO containing cultures throughout the times shown.

Fig. 2. Appearance of phospholipase activity in DMSO treated U937 cells. U937 cells were grown as described in Fig. 1 in the absence (●) or presence (o) of 1.5% DMSO. At the times indicated, cells were assayed for 2 minutes for the presence of phospholipase activity as described in Materials and Methods in the presence of 10 uM A_{23187}. Incorporation of [1-^{14}C]arachidonic acid into cellular lipids was greater than 80% in control and 60% in DMSO-treated cells after overnight labelling. The amount of esterified [1-^{14}C]arachidonic acid in the assays was 8.0×10^4 to 1.7×10^5 dpm/10^6 cells for control and 1.1×10^5 to 2.5×10^5 dpm/10^6 cells for DMSO-treated cells. Assays contained 4 - 10 x 10^6 cells. Results are reported as the per cent of esterified arachidonic acid released.

concentration of the ionophore of approximately 10 uM. Control cells do not release significant amounts of arachidonic acid from their endogenous phospholipids at concentrations of the calcium ionophore as high as 20 uM.

Calcium ionophore induced release of arachidonic acid is a rapid process. Maximal release occurs in two minutes with the quantity of unesterified arachidonic acid declining at longer times (Fig. 4). This decline is apparently due to the re-esteri-

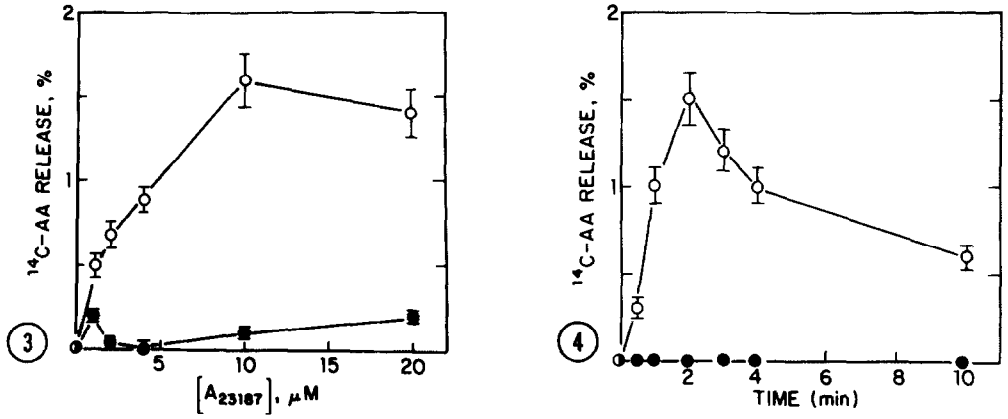


Fig. 3. Calcium ionophore concentration dependence of arachidonic acid release, Control (●) and 4 day DMSO-treated (o) U937 cells were assayed for their ability to release endogenous esterified [1-^{14}C]arachidonic acid in the presence of 1 mM $CaCl_2$ and various concentrations of the calcium ionophore A_{23187}, as described in Fig. 2.

Fig. 4. Time course of arachidonic acid release. U937 cells treated for 4 days with DMSO were assayed in the presence (o) or absence (●) of 10 uM A_{23187} in the presence of 1 mM $CaCl_2$ as described in Fig. 2.

fication of the unsaturated fatty acid since negligible metabolism of arachidonic acid to prostaglandins, HHT, 5-HETE or LTB_4 is observed (data not shown). Insignificant quantities of unesterified arachidonic acid are detected in the absence of A_{23187} (Fig. 4).

Ionophore induced release of arachidonic acid is dependent upon exogenous calcium ion. In the absence of free calcium in the assay medium, no phospholipase activity is observed in the absence or presence of 10 uM A_{23187} (Fig. 5). However, as the calcium ion concentration is increased, significant phospholipase stimulation by A_{23187} occurs. Optimal release of arachidonic acid occurs with free calcium ion concentrations between 0.5 and 2.0 mM (Fig. 5).

Antigen-antibody complexes are natural stimulators of macrophage function (12,13,15,17). As a measure of the ability of DMSO-treated but not control U937 cells to express activities consistent with those of normal macrophages, cells were tested

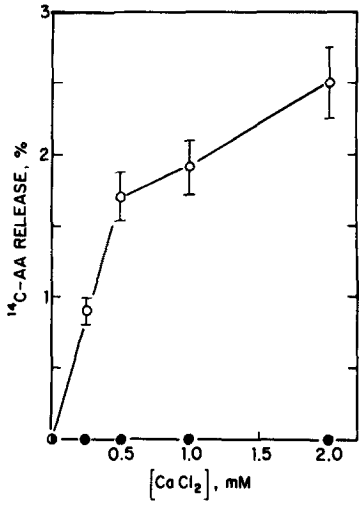


Fig. 5. Dependence of arachidonic acid release on exogenous calcium ion. U937 cells treated for 4 days with DMSO were assayed as described in Fig. 2 in the presence (o) or absence (•) of 10 uM A_{23187} for 2 minutes at various concentrations of exogenous calcium ion.

for their response to IgG-anti-IgG complex formation. As illustrated in Figure 6, DMSO treated cells, when exposed first to human IgG and then anti-human IgG, release arachidonic acid from their endogenous phospholipids in a rapid burst of phospholipase activity. The formation of the IgG-anti-IgG complex is necessary for the expression of this activity, since IgG alone does not cause release (Fig. 6). In addition, control cells - those not grown in DMSO - do not release arachidonic acid from their endogenous lipids when exposed to IgG and anti-IgG (data not shown).

DISCUSSION: Arachidonic acid metabolism in macrophages has been the subject of extensive research recently (8-15, 17). An activatable phospholipase, which releases the unsaturated fatty acid from membrane phospholipids, has been described (13, 18-20). In addition, macrophages are known to metabolize unesterified arachidonic acid via the cyclo-oxygenase and lipoxygenase pathways (8-10,12,15,18-20).

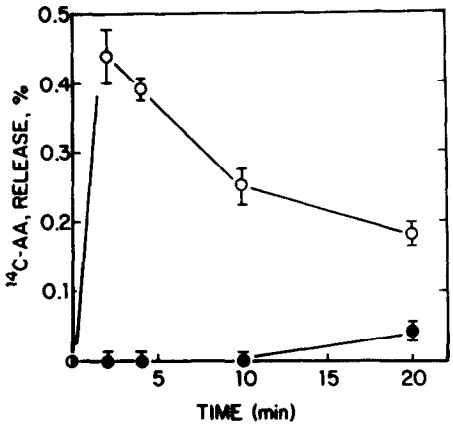


Fig. 6. IgG-anti-IgG stimulated arachidonic acid release. Four day DMSO-treated U937 cells were preincubated for one hour at 37° with 1 mg/ml human IgG (Sigma) in RPMI 1640 and then washed and resuspended in 50 mM Tris-HCl buffer, pH 7.4, containing 100 mM NaCl, 1 mM $CaCl_2$ and 1 mM glucose. The preincubated cells were assayed in the presence (o) or absence (●) of 1 mg/ml anti-human IgG (Sigma) for the times indicated as described in Materials and Methods.

The present study describes the appearance of an arachidonic acid metabolic activity, phospholipase, in U937 cells which have been induced to differentiate by exposure to dimethyl sulfoxide. The absence of a phospholipase activity in undifferentiated U937 cells would prevent metabolism of endogenous esterified arachidonic acid via cyclo-oxygenase or lipoxygenase and functional activities dependent upon these metabolic products. For example, the ability to respond to immunoglobulin mediated signals by the release and subsequent metabolism of arachidonic acid, a characteristic of normal macrophages (12,13,20), is only present in the DMSO cultured cells. While specific surface antigens and peptide receptors have been shown to be induced by the growth of U937 cells in conditioned medium, DMSO or dibutyryl cAMP (2,4-6,21), this is the first report of the induction of an arachidonic acid metabolizing enzyme in these cells. It has been previously reported that while exogenously added arachidonic acid could be metabolized to prostaglandins by control U937 cells, challenge of these cells with zymosan, opsonized zymosan, phorbol

myristate acetate, heat aggregated IgG or calcium ionophore A_{23187} failed to stimulate PGE_2 synthesis from endogenous arachidonic acid (22). The lack of phospholipase activity in control cells and its induction by DMSO (Fig. 2) explains these findings. The fact that DMSO-treated but not control U937 cells can respond to activators such as the calcium ionophore A_{23187} and IgG-anti-IgG complex formation suggests that the U937 cell line may serve as a useful model for studying the role of arachidonic acid metabolism in human macrophage function.

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